

CLAIMS

1. A process for production of an alcohol product comprising the sequential steps of:
 - (a) providing a slurry comprising water and granular starch,
 - 5 (b) holding said slurry in the presence of an acid alpha-amylase and a glucoamylase at a temperature of 0°C to 20°C below the initial gelatinization temperature of said granular starch for a period of 5 minutes to 12 hours,
 - (c) holding said slurry in the presence of an acid alpha-amylase and a glucoamylase and a yeast at a temperature between 10°C and 35°C for a period of 20 to 250
 - 10 hours to produce ethanol and,
 - (d) optionally recovering the ethanol.
2. The process according to claim 1, wherein the product is fuel ethanol, potable ethanol and/or industrial ethanol.
- 15 3. The process according to any of the preceding claims wherein the temperature under step (c) is between 28°C and 36°C, such as between 29°C and 35°C, such as between 30°C and 34°C, such as around 32°C.
4. The process according to claim 1, wherein the product is a beer.
5. The process according to any of claims 1 or 4 wherein the temperature under step (c) is between 11°C and 17°C, such as between 12°C and 16°C, such as between 13°C and 20 15°C, such as around 14°C.
6. The process according to any of the preceding claims wherein the activities of acid alpha-amylase and glucoamylase is added in step (b) and/or (c) in a ratio of between 0.30 and 5.00 AFAU/AGU.
- 25 7. The process according to any of the preceding claims wherein the acid alpha-amylase is an acid fungal alpha-amylase.
8. The process according to any of the preceding claims wherein the acid fungal alpha-amylase is obtained from a strain of *Aspergillus*, preferably a strain of *Aspergillus niger* or a strain of a strain of *Aspergillus oryzae*.
- 30 9. The process according to any of the preceding claims wherein the acid alpha-amylase is an

acid alpha-amylase having an amino acid sequence which has at least 70%, preferably at least 75%, 80%, 85% or at least 90%, e.g. at least 95%, at least 97%, at least 98%, or at least 99% homology to SEQ ID NO:1.

10. The process according to any of the preceding claims wherein the glucoamylase is obtained
5 from a strain of *Aspergillus*, *Talaromyces* or *Clostridium*.
11. The process according to any of the preceding claims wherein the glucoamylase is obtained
from a strain of *Aspergillus niger*.
12. The process according to any of the preceding claims wherein the acid alpha-amylase is an
acid bacterial alpha-amylase.
- 10 13. The process according to any of the preceding claims wherein the acid alpha-amylase is
derived from a strain of *B. licheniformis*, *B. amyloliquefaciens*, and *B. stearothermophilus*
alpha-amylase.
- 15 14. The process according to any of the preceding claims wherein the acid bacterial alpha-
amylase is derived from a strain of *B. licheniformis*, *B. amyloliquefaciens*, and *B.*
stearothermophilus alpha-amylase.
- 15 15. The process according to any of the preceding claims wherein the acid bacterial alpha-
amylase is derived from a strain of *Bacillus stearothermophilus*, having the mutations I181*
+ G182* + N193F compared to the wild type amino acid sequence set forth in SEQ ID
NO:2.
- 20 16. The process according to any of the preceding claims wherein the acid bacterial alpha-
amylase is a hybrid alpha-amylase comprising the 445 C-terminal amino acid residues of
the *Bacillus licheniformis* alpha-amylase set forth in SEQ ID NO:3 and the 37 N-terminal
amino acid residues of the alpha-amylase derived from *Bacillus amyloliquefaciens* set forth
in SEQ ID NO:4, having the substitution G48A + T49I + G107A + H156Y + A181T +
25 N190F + I201F + A209V + Q264S.
17. The process according to any of the preceding claims wherein the acid bacterial alpha-
amylase is a alpha-amylase having the amino acid sequence set forth in SEQ ID NO:4,
having the mutations H154Y, A181T, N190F, A209V, Q264S, and/or deletion of two
residues between positions 176 and 179, preferably deletion of E178 and G179.
- 30 18. The process according to any of the preceding claims, wherein the acid alpha-amylase

activity is present in an amount of 50-500 AFAU/kg of DS.

19. The process according to any of the preceding claims wherein the glucoamylase activity is present in an amount of 20-200 AGU/kg of DS.
20. The process according to any of the preceding claims, wherein the ratio between acid alpha-amylase activity and glucoamylase activity is between 0.35 and 5.00 AFAU/AGU.
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21. The process according to any of the preceding claims, wherein step (b) and/or step (c) is performed in the presence of an enzyme activity selected from the list consisting of xylanase, cellulase and phytase.
22. The process of the preceding claim, wherein the starch slurry has 5-60% DS granular starch, preferably 10-50% DS granular starch, more preferably 20-40% DS, especially around 30% DS granular starch.
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23. The process of any of the preceding claims, wherein the pH during step (b) and/or (c) is in the range of 3.0 to 7.0, preferably from 3.5 to 6.0, or more preferably from 4.0-5.0, such as from 4.3 to 4.6.
- 15 24. The process of any of the preceding claims, wherein the granular starch is obtained from tubers, roots, stems, fruits, seeds or whole grain.
25. The process of any of the preceding claims, wherein the granular starch is obtained from corn, cobs, wheat, barley, rye, milo, sago, cassava, manioc, tapioca, sorghum, rice or potatoes.
- 20 26. The process of any of the preceding claims, wherein the granular starch is obtained from cereals.
27. The process of any of the preceding claims, wherein the granular starch is obtained from dry milling or wet milling of whole grain.
28. The process according to any of the preceding claims wherein the holding time under step (b) is from 5 minutes to 12 hours, preferably from 10 minutes to 6 hours, more preferably from 15 minutes to 3 hours, even more preferably from 20 minutes to 1½ hour, such as from 30 minutes to 1 ¼ hour, from 40 to 70 minutes, and even from 50 to 60 minutes.
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29. The process according to any of the preceding claims wherein the holding time under step

- (c) is from 25 to 190 hours, preferably from 30 to 180 hours, more preferably from 40 to 170 hours, even more preferably from 50 to 160 hours, yet more preferably from 60 to 150 hours, even yet more preferably from 70 to 140 hours, and most preferably from 80 to 130 hours.
- 5 30. The process according to any of the preceding claims wherein the temperature under step
 (b) is from 45°C to 75°C.
- 10 31. An enzyme composition comprising acid alpha-amylase activity and glucoamylase activity in
 a ratio of between 0.30 and 5.00 AFAU/AGU, wherein an additional enzyme activity is
 present; said enzyme activity is selected from the list consisting of cellulase, xylanase and
 phytase.
- 15 32. Use of an enzyme composition according to the preceding claim in an alcohol product
 process or a starch hydrolysis process.
- 15 33. Use of an enzyme composition comprising acid alpha-amylase activity and glucoamylase
 activity in a ratio of between 0.30 and 5.00 AFAU/AGU, in an alcohol product process
 comprising enzymatic hydrolysis of granular starch.
34. A mashing process comprising the application of an acid alpha-amylase.
35. The process according to the preceding claim, wherein the acid alpha-amylase is derived
 from a fungus within the *Aspergillus*, preferably from *A. niger*.
- 20 36. The process according to any of the preceding claims, wherein the acid alpha-amylase has
 at least 50%, at least 60%, at least 70%, at least 80%, at least 90% homology to the amino
 acid sequence shown in SEQ ID NO:1
- 25 37. The process according to any of the preceding claims, comprising:
 a. forming a mash comprising between 5% and 100% barley malt (w/w of the grist);
 b. prior to, during or after a) adding at least one enzyme selected from the list
 comprising: a protease (E.C. 3.4.), cellulase (E.C. 3.2.1.4) and a maltose generating
 enzyme.
 c. attaining within 15 minutes of a) an initial incubation temperature of at least 70°C;
 d. following c) incubating the mash at a temperature of at least 70°C for a period of
 time sufficient to achieve an extract recovery of at least 80%; and,

e. separating the wort from the spent grains.